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Formylated Chemotactic Peptides Can Mimic the Secondary, Provoking Endotoxin Injection in the Generalized Shwartzman Reaction

Fehr, J ; Dahinden, C ; Russi, R

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Legend. Female New Zealand White rabbits of 2.0–2.5 kg weight were prepared for induction of left-side bacterial endocarditis as previously described [1], with the polyethylene catheter left in place throughout the experiment. After placement of the catheter rabbits were randomly assigned to receive either antibiotic therapy or no treatment. Forty-eight hours after placement of the catheter prophylactic therapy was begun. All animals in the treatment groups were given *N*-formimidoyl thienamycin 15–20 min before the iv injection of 1 ml of an 18 hr culture of the test organism. Groups of treated animals were then assigned to receive one, two, or three additional doses of antibiotic every 2.5 hr after infection. Each dose was of 33 mg *N*-formimidoyl thienamycin/kg body weight, given sc. Forty-eight hours after the last dose of antibiotic, animals were killed, vegetations were excised, weighed, and homogenized in 2.0 ml of brain-heart infusion (BHI) broth. Undiluted homogenate and 10-fold dilutions were incorporated into 20 ml BHI-agar pour plates in duplicate. After incubation at 37 °C for 48 hr, the number of cfu/g of vegetation was calculated. Positive vegetations were defined as ≥ 1 cfu recovered from the vegetation.

Inocula of 1 ml of organisms (numbers shown in table) were prepared from 18 hr cultures in BHI broth. Four strains of *Streptococcus faecalis* were used; two (EBC-2 and EIR, kindly provided by R.C. Moellering, Jr.) were streptomycin-resistant ($>6,400 \mu\text{g/ml}$) and two (B-9 and 4037) were streptomycin-susceptible. Two strains of *Staphylococcus aureus* were tested, one of which (Berman) was methicillin-resistant ($>100 \mu\text{g/ml}$). Two strains of *Streptococcus sanguis* and one strain of *Streptococcus intermedius* were also evaluated. Mortality during the course of the experiment occurred only in the untreated animals infected with *S. aureus* strains (50% for each strain) and in one untreated animal inoculated with *S. sanguis* I. MIC and MBC for *N*-formimidoyl thienamycin against each strain was ascertained with the macrodilution technique.

Organism (inoculum/ml)		MIC/MBC ($\mu\text{g/ml}$)	Number of doses of antibiotic				
			1 (Preinfection)	2	3	4	No treatment
<i>Streptococcus faecalis</i>							
Strain B-9	(1.2×10^7)	0.78/ ≥ 50	2/8	1/7	0/7	0/7	9/9
4037	(1.1×10^7)	0.78/ ≥ 50	...	2/7	...	1/9	8/8
EBC-2*	(1.3×10^7)	0.78/ ≥ 50	1/8	0/8	0/7	0/7	7/7
EIR*	(1.0×10^7)	0.78/ ≥ 50	...	2/9	...	0/9	10/10
<i>Staphylococcus aureus</i>							
Strain 2776	(2.6×10^6)	0.05/0.20	4/8	0/8	1/8	0/8	4/4 [‡]
Berman [†]	(1.3×10^6)	1.56/100	...	0/9	...	0/10	5/5 [‡]
Other <i>Streptococcus</i> species							
<i>S. sanguis</i> I	(2.6×10^7)	0.10/0.20	9/9	8/8	6/8	6/7	7/7
<i>S. sanguis</i> II	(1.5×10^7)	0.10/0.39	1/7	1/8	0/8	0/8	8/8
<i>S. intermedius</i>	(7.9×10^7)	0.10/0.39	5/6	6/7	5/7	3/7	8/8
<i>S. intermedius</i>	(1.7×10^7)						
(repeat of above strain)			8/8	6/7	6/7	5/8	9/9

* Streptomycin-resistant.

[†] Methicillin-resistant.

[‡] Culture of live animals only; 50% mortality prior to sacrifice.

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Formylated Chemotactic Peptides Can Mimic the Secondary, Provoking Endotoxin Injection in the Generalized Shwartzman Reaction

COLLEAGUES: The generalized Shwartzman reaction, the toxic reaction that occurs when two sequential iv injections of endo-

toxin are given 24 hr apart, is a powerful, although still enigmatic animal model with which to study the plethora of *in vivo* biologic effects of endotoxin. It is known that the preparative state can be brought about in ways and by agents other than by endotoxin (i.e., pregnancy, pretreatment with corticosteroids) and it has recently been shown that staphylococcal pyrogenic exotoxin C dramatically enhances host susceptibility to lethal shock caused by endotoxin [1]. For these reasons we addressed ourselves to the question of whether *N*-formyl methionyl peptides that represent synthetic analogues of granulocyte-activat-

Please address requests for reprints to Dr. J. Fehr, Clinical Hematology, Department of Medicine (Clinic) CH5, University Hospital, Rämistrasse 100, CH-8091 Zurich, Switzerland

Legend. Experiments on possible interchangeability of endotoxin and *N*-formylated methionine-leucine-phenylalanine (fMLP) in the generalized Shwartzman reaction.

Animal groups (rabbits)	Preparative injection (neutropenia)*	Provocative injection (neutropenia)	No. dead/total no. of rabbits treated
A	Endotoxin (p*)	Endotoxin (p)	3/3
B	fMLP (t)	Endotoxin (p)	0/8†
C	Endotoxin (p)	fMLP (p)	6/9†
D	fMLP (t)	fMLP (t)	0/3

NOTE. Rabbits that weighed 3–4 kg were anesthetized with Hypnorm® (Philips Duphar, Amsterdam). Endotoxin (*Escherichia coli* 026:B6; Difco, Detroit), 380 µg/kg of body weight, and fMLP (Bachem AG, Bubendorf, Switzerland), 2 µmol/animal, were administered through ear veins. Blood for granulocyte counts was drawn from the carotid artery through an inserted cannula at 0, 15, 60, and 120 min. Animals died in less than 12 hr. The results in group D confirm eight years of personal experience of fMLP injections into rabbits.

* p = persistent neutropenia (circulating granulocytes remained below 15% for more than 120 min); t = transient neutropenia (circulating granulocytes were below 15% at 15 min, reached pre-injection values at 60 min, and were overshooting at 120 min).

† Mortality between groups B and C is significantly different ($P < .01$ by Fisher's exact *t* test).

ing bacterial products [2] (as distinct from lipopolysaccharides) might act as substitutes for either the preparative or the provocative injection of endotoxin. The main rationale for performing these experiments was that both endotoxin and formylated peptides provoke a precipitous and profound fall in the number of circulating granulocytes. Our results clearly show that *N*-formylated methionine-leucine-phenylalanine (fMLP) is a deleterious substance in animals that have been prepared with endotoxin. In contrast, preparation of animals with fMLP does not induce increased susceptibility to endotoxin. The mechanisms responsible for this phenomenon are presently unknown. Since endotoxin and fMLP possess similar granulocyte activating potencies [3], we suggest that endotoxin-induced endothelial damage may play an essential role: if fMLP-activated, hyperadhesive [4] granulocytes encounter endothelial cells that have been injured by endotoxin (an injury that does not occur with fMLP alone), the result is a deleterious sequence of events (e.g., persistent neutropenia; see table). In contrast, if granulocytes activated by either endotoxin or fMLP encounter native, undamaged endothelium, subsequent alterations remain limited.

In conclusion, our results show that endotoxin and other bacterial products can have a pathomechanistic role in enhance-

ment of susceptibility to lethal endotoxin shock. If similar interchangeability of endotoxin and fMLP analogues are realized in humans, stimulator-directed therapeutic interventions may be desirable. Phenylbutazone has been recognized as a specific and clinically applicable antagonist for fMLP-activation of granulocytes [4], so effective intervention may be feasible.

J. FEHR, C. DAHINDEN, R. RUSSI

University Hospital, Zurich, Switzerland

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Mycoplasma pulmonis in the Brains of Rodents: A Rare Event

TO THE EDITOR: We were interested in the article by Hill [1] in which she reported that in all rats tested, intranasal inoculation of *Mycoplasma pulmonis* organisms lead to infection of the brain 10–14 days later. This communication followed a previous one [2] in which Hill reported recovery of *M. pulmonis* from the brains of all mice that either harbored the organisms in the

nasopharynx naturally or that had been inoculated via the nose. These results for mice were in accord with the report by Saito et al. [3] of very rapid infection of the brains of mice by *M. pulmonis* after the mice had been placed in contact with others that had been infected nasally. In our experience, natural transmission from nose to nose in mice does not occur readily, and we found [4] that recovery of *M. pulmonis* from the brain was a rare event. This was the reason why the report of recovery of *M. pulmonis* from the brains of all rats so soon after inoculation, apart from being inherently remarkable, sufficiently stimulated our interest to convince us to undertake experiments of a similar kind. Five- to six-week-old Lac:P male and female rats (the same strain as that used by Hill) were anesthetized by ip inoculation of Hypnorm® (Crown Chemical, U.K.; 0.002

Please address requests for reprints to Dr. D. Taylor-Robinson, Division of Sexually Transmitted Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, Great Britain.